APPENDIX 4: STANDARD OPERATING PROCEDURES for the TREATMENT of WILD HORSES with a PORCINE ZONA PELLUCIDA CONTRACEPTIVE VACCINE

1. PURPOSE

The BLM currently employs a one-injection contraceptive vaccine that provides one or 2 years of infertility, with a return to fertility thereafter. Because wild horses are best accessible for injection when they are rounded up into a field corral system, BLM would like to increase the contraceptive duration to 3-4 years to more closely coincide with their present round-up/adoption program schedules. A 3-4 year vaccine will also broaden the options for fertility control in general. The proposed research will involve: 1) a lab study to formulate the 3-4 year vaccine and do preliminary testing, and 2) a test of the vaccine's effectiveness (via pregnancy testing) in captive mares at a BLM facility. Preferably, both the immune response (historically a reasonably reliable indicator of fertility level) and pregnancy rate should be determined.

II. PARTICIPANTS

Principal Investigator:	John Turner, Jr., PhD. Medical College of Ohio, Toledo, Ohio.
Project Inspector:	Linda Coates-Markle, National Research Coordinator, Wild Horse and Burro Program. BLM, Billings Field Office, Mt.
Assistance Representative:	Dean Bolstad, BLM Wild Horse and Burro Program Lead, Reno, Nv.
Vaccine Preparation:	Robin Lyda, The Science and Conservation Center, ZooMontana, Billings, Mt.
Pellet Production:	Douglas Flanagan, PhD., University of Iowa,
Project Veterinarian:	Marvin Hamann, DVM, Contract Veterinarian, Wild Horse Inmate Program, East Cañon Correctional Complex outside Cañon City, Colorado.

III. RESEARCH PLAN PROTOCOL

Materials and Methods - in vivo Option #1 (with Captive Breeding)

For this study access to 38 captive wild mares (preferably 6-18 years of age) will be necessary in a BLM facility that is conducive to a routine venous blood sampling procedure. All animal studies will be performed under auspices of MCO Animal Care and Use protocol (#100742) and current BLM horse-care standards, which are currently in place. While the new formulation will differ from previously tested pellets in polymer/ingredient ratios, both polymer type and pellet active ingredients will be the same as those in previously tested pellets except that

amounts of PZP and AQ-21 will be greater. Thus, safety testing of the new pellets prior to use in captive mares should not be necessary.

Twenty mares will be immunized with a one-injection PZP vaccine having a presumptive duration of 3-4 years, and 9 mares will be given the established reference vaccine of PZP (65 μg)/Freund's Complete Adjuvant (FCA) emulsion with a 1-month booster of PZP/Freund's Incomplete Adjuvant (FIA). For comparison more closely with the single-injection, 3^+ PZP vaccine these 9 mares will be given an annual booster at 12 and 24 months. This regimen has been shown to sustain infertility annually in mares (Kirkpatrick et al., 1992) and will thus serve as a reliable comparator for the 3^+ PZP vaccine. All mares will be blood sampled by a veterinarian or qualified technician every 2 weeks for 6 weeks and thereafter at 5, 10, 15, 17, 18, 19, 20, 21, 22, and 24 months (days 28, 56, 112, 168, 216, 300, 450, 510, 570, 630, and 720) for anti-PZP titer analysis. Titers will be determined by an established ELISA. Since previous studies have shown that maintenance of antibody titers above 60% of positive reference control titers has been consistently associated with infertility in mares, maintenance of such titers throughout the proposed study in mares given 3^+ PZP vaccine will indicate high likelihood of infertility. Nonetheless, final assessment of the 3^+ PZP vaccine will require a breeding test to demonstrate inhibition of fertility for 3 continuous years. Performance of such a test in the same captive mares being titer-tested would be the ideal and most cost effective means of testing fertility. Specifically, the 29 PZP-treated mares above and 8-10 untreated mares will be exposed to 4 stallions of proven fertility in a pasture setting from May through July of the third year (to test effectiveness) and the fourth year (to test reversibility/4^{th} year effectiveness). Pregnancy will be determined by fecal hormone analysis of fecal samples collected in November of years 3 and 4.

IV. PROCEDURES

A. Vaccine preparation and shipment: Vaccine would be prepared under the supervision of Robin Lyda, Science and Conservation Center (SCC), Billings, Mt. Pellet production for the controlled-release version of the vaccine is to take place at the University of Iowa under the direction of Dr. Douglas Flanagan. All vaccine products are to be shipped to the Wild Horse Inmate Program, East Cañon Correctional Complex outside Cañon City, Colorado on dry ice (as necessary), under Food and Drug Administration authority (Investigational New Animal Drug exemption No.8857 G0002 & 0003). FDA form "Notice of Drug Shipment" would be completed for each shipment of the PZP vaccine and filed in the offices of the Science and Conservation Center at ZooMontana, Billings, Mt., or suitable and appropriate research facilities.

B. Selection of subject animals and withdrawal of animals from the study: Animals to be treated will have been previously freezemarked by BLM personnel with the BLM Alpha Angle system. All animals selected for treatment are female and at least two-years old. Animals are selected on the basis of body condition (at least fair to good), and the lack of current pregnancy or a foal at their side. Animals with obvious signs of illness or poor health will not be included in the study. Animals will be withdrawn from the study if in the opinion of the Project Veterinarian and/or the Project Manager they develop health or behavioral conditions that would be exacerbated by continuation of the

research, or continuation of the research would result in health problems such as but not limited to serious systemic or localized reactions, injury or death.

C. Squeeze-chute delivery of contraceptive vaccine: The inoculation of mares held within a squeeze chute would consist of a liquid dose of PZP vaccine as a primer and/or a liquid or time released (pellet portion) of the drug as a booster. Delivery of the vaccine would be by means of syringe or dart with a 12 gauge needle or 1.5" barbless needle respectfully. 0.5 cc of the PZP vaccine would be emulsified with 0.5 cc of adjuvant (a compound that stimulates antibody production) and loaded into the delivery system. The pellets would be placed in the barrel of the syringe or dart needle and would be injected with the liquid. Animals which have never been treated would be treated with PZP + Freund's Complete (or Freund's Modified) adjuvant, while animals, which have been previously treated would be given PZP + Freund's Incomplete adjuvant. Only hip or gluteal muscle regions of the horse are acceptable targets. Only trained personnel would mix and/or administer the vaccine.

The time release approach incorporates the PZP into a non-toxic, bio-degradable material which can be formed into small pellets. The pellets are injected with the liquid and are designed to release PZP at several points in time much the way time-release cold pills work. This formulation would be delivered as an intramuscular injection by a jabstick syringe, into the mares in the working chute. Upon impact the liquid in the chamber would be propelled into the muscle along with the pellets. This delivery method has been used previously to deliver inmmunocontraception vaccine with acceptable results. Such a vaccine may permit a single injection to cause up to two years of contraception at approximately 90% efficiency.

D. Record keeping: BLM, MCO and/or SCC personnel and/or all research personnel involved with the project would maintain records for the identification of all horses to be vaccinated or used for control purposes. These records would be used to meet FDA regulations for use of the vaccine under the existing INAD. Each horse vaccinated will be permanently identified by a unique BLM freezemark identification number. For each horse vaccinated, the following information will be recorded:

1. identification of vaccine administrator
2. date of inoculation
3. size of PZP dose
4. type of adjuvant
5. type of delivery system
6. precise site of inoculation (right or left side of hip)

Additionally, other observations regarding estrous behavior, swelling at the site of injection, presence of abscesses or other concerns, and any other pertinent information collected by researchers or the Wild Horse and Burro Specialist would be maintained by the BLM. The ideal time for monitoring of initial localized injection reactions would be between 24 and 48 hours post-vaccination. During this time frame each horse will be examined in a chute and potential injection sites on the left and right sides palpated by hand.

The area of injection will be scored by a trained observer using the following scale: 0=no visible or palpable reaction; 1=some swelling visible or palpable; 2=swelling readily apparent, somewhat painful to palpation; 3= obvious swelling or pain on visualization/palpation, some lameness variably present; 4= obvious swelling, very painful to the touch or consistent lameness present. The presence or absence of other systemic or localized signs of reactions (abscessation, obvious swelling) will be recorded as observed following visual inspection and treated as indicated by the attending veterinarian. Injection site and systemic reactions will be treated (including the judicious use of phenylbutazone for pain relief and as an anti-inflammatory) under the direction of the attending veterinarian. A record of these observations and any treatments required will be maintained as part of the study records by the BLM.

E. Veterinary emergencies – General: The project veterinarian (or an appointed and certified replacement) will be on site during any handling and vaccinating of mares while in a captive setting. It is expected that the project veterinarian will be on-call during other times of the study. All incidences of veterinary or animal health concerns and animal treatment will be recorded and maintained as part of the records for the study by the BLM.

The project veterinarian will have oversight on all clinical practices associated with the study. This may include but not be limited to blood draws, the assessment and treatment of abscesses and /or granulomas as well as any diseases that may develop during the course of the study or any injuries sustained by the animals during handling procedures. Appropriate treatment procedures will be determined by the project veterinarian on a case by case basis.

F. Blood samples/recovery of ovaries: An attempt to recover blood samples for antibody analysis and to recover ovaries for determination of ovarian effects shall be carried out opportunistically. In the unlikely event that a female horse must be euthanized for humane reasons, a blood sample would be immediately collected in a red top10 cc tube. The sample would be sent to the Project Veterinarian where the serum would be harvested and stored frozen. If at all possible, at least one and preferably both, ovaries would be excised and placed in 10% buffered formalin, for histological examination.

G. **Euthanasia of horses:** Guidelines for the euthanasia of wild horses or burros contained in BLM Manual 4730-Destruction of Wild Horses and Burros and Disposal of their Remains and BLM Instruction Memorandum No. 2001-165, Euthanasia of Wild Horses and Burros will be followed. When ordered by the BLM Authorized Officer, euthanasia will be performed in accordance with recommendations from the 2000 Report of the AVMA Panel on Euthanasia (JAVMA.2001;218:669-696). Intravenous administration of a lethal dose of a barbituric acid derivative (for example Socumb-6GR, containing 389mg/ml pentobarbital sodium, administered at a dosage of 1ml/10lbs) will be the preferred method of euthanasia. When consideration of the wild nature of the horse or burro suggests that an alternative method may result in less fear and anxiety,

and be more rapid, painless or humane, or when under field conditions, the possible consumption of carcasses of euthanatized animals by predators or scavengers cannot be avoided with certainty, euthanasia by gunshot may be performed.

H. Necropsies in captive settings: In the unlikely event of the death of mares being used for studies in captive settings, a full necropsy will be performed, including gross and histologic examination of all major organ systems. The procedure will be carried out by a board-certified veterinary pathologist when possible with a full report being prepared for distribution to the Research Team and maintained as part of the study records by BLM.

I. Media relations: All requests by the media (verbal, written or electronic), must ultimately pass through the Principal Investigator or Project Inspector or their designate, and the decision to release information related to the project shall rest with the Principal Investigator and/or BLM.